



<110> Leeflang, Chris
Van der Kleij, Wilhelmus A.H.

<120> Methods for Site-Directed Mutagenesis
and Targeted Randomization

<130> GC787-2-US

<140> US 10/541,737

<141> 2005-07-08

<150> PCT/US04/01334

<151> 2004-01-16

<150> US 60/440,792

<151> 2003-01-16

<160> 7

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 275

<212> PRT

<213> Bacillus amyloliquefaciens

<400> 1

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His	Ser	Gln	Gly	Tyr	Thr	Gly	Ser	Asn	Val	Lys	Val	Ala	Val	Ile	Asp
		20						25					30		
Ser	Gly	Ile	Asp	Ser	Ser	His	Pro	Asp	Leu	Lys	Val	Ala	Gly	Gly	Ala
		35					40					45			
Ser	Met	Val	Pro	Ser	Glu	Thr	Asn	Pro	Phe	Gln	Asp	Asn	Asn	Ser	His
	50					55					60				
Gly	Thr	His	Val	Ala	Gly	Thr	Val	Ala	Ala	Leu	Asn	Asn	Ser	Ile	Gly
65					70					75				80	
Val	Leu	Gly	Val	Ala	Pro	Ser	Ala	Ser	Leu	Tyr	Ala	Val	Lys	Val	Leu
				85					90					95	
Gly	Ala	Asp	Gly	Ser	Gly	Gln	Tyr	Ser	Trp	Ile	Ile	Asn	Gly	Ile	Glu
		100						105					110		
Trp	Ala	Ile	Ala	Asn	Asn	Met	Asp	Val	Ile	Asn	Met	Ser	Leu	Gly	Gly
		115					120					125			
Pro	Ser	Gly	Ser	Ala	Ala	Leu	Lys	Ala	Ala	Val	Asp	Lys	Ala	Val	Ala
		130				135					140				
Ser	Gly	Val	Val	Val	Val	Ala	Ala	Ala	Gly	Asn	Glu	Gly	Thr	Ser	Gly
145					150					155				160	
Ser	Ser	Ser	Thr	Val	Gly	Tyr	Pro	Gly	Lys	Tyr	Pro	Ser	Val	Ile	Ala
			165						170					175	
Val	Gly	Ala	Val	Asp	Ser	Ser	Asn	Gln	Arg	Ala	Ser	Phe	Ser	Ser	Val
			180					185					190		
Gly	Pro	Glu	Leu	Asp	Val	Met	Ala	Pro	Gly	Val	Ser	Ile	Gln	Ser	Thr
		195				200					205				
Leu	Pro	Gly	Asn	Lys	Tyr	Gly	Ala	Tyr	Asn	Gly	Thr	Ser	Met	Ala	Ser
		210				215					220				
Pro	His	Val	Ala	Gly	Ala	Ala	Ala	Leu	Ile	Leu	Ser	Lys	His	Pro	Asn
225					230					235					240

Trp Thr Asn Thr Gln Val Arg Ser Ser Leu Glu Asn Thr Thr Thr Lys
 245 250 255
 Leu Gly Asp Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala
 260 265 270
 Ala Ala Gln
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<210> 2
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 <212> PRT
 <213> Bacillus subtilis

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 1 5 10 15
 His Ser Gln Gly Tyr Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp
 20 25 30
 Ser Gly Ile Asp Ser Ser His Pro Asp Leu Asn Val Arg Gly Gly Ala
 35 40 45
 Ser Phe Val Pro Ser Glu Thr Asn Pro Tyr Gln Asp Gly Ser Ser His
 50 55 60
 Gly Thr His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly
 65 70 75 80
 Val Leu Gly Val Ser Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu
 85 90 95
 Asp Ser Thr Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu
 100 105 110
 Trp Ala Ile Ser Asn Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly
 115 120 125
 Pro Thr Gly Ser Thr Ala Leu Lys Thr Val Val Asp Lys Ala Val Ser
 130 135 140
 Ser Gly Ile Val Val Ala Ala Ala Gly Asn Glu Gly Ser Ser Gly
 145 150 155 160
 Ser Thr Ser Thr Val Gly Tyr Pro Ala Lys Tyr Pro Ser Thr Ile Ala
 165 170 175
 Val Gly Ala Val Asn Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Ala
 180 185 190
 Gly Ser Glu Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr
 195 200 205
 Leu Pro Gly Gly Thr Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Thr
 210 215 220
 Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Thr
 225 230 235 240
 Trp Thr Asn Ala Gln Val Arg Asp Arg Leu Glu Ser Thr Ala Thr Tyr
 245 250 255
 Leu Gly Asn Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala
 260 265 270
 Ala Ala Gln
 275

<210> 3
 <211> 274
 <212> PRT
 <213> Bacillus licheniformis

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			20					25					30				
Thr	Gly	Ile	Gln	Ala	Ser	His	Pro	Asp	Leu	Asn	Val	Val	Gly	Gly	Ala		
		35					40					45					
Ser	Phe	Val	Ala	Gly	Glu	Ala	Tyr	Asn	Thr	Asp	Gly	Asn	Gly	His	Gly		
	50				55					60							
Thr	His	Val	Ala	Gly	Thr	Val	Ala	Ala	Leu	Asp	Asn	Thr	Thr	Gly	Val		
65					70					75					80		
Leu	Gly	Val	Ala	Pro	Ser	Val	Ser	Leu	Tyr	Ala	Val	Lys	Val	Leu	Asn		
			85					90						95			
Ser	Ser	Gly	Ser	Gly	Ser	Tyr	Ser	Gly	Ile	Val	Ser	Gly	Ile	Glu	Trp		
		100						105					110				
Ala	Thr	Thr	Asn	Gly	Met	Asp	Val	Ile	Asn	Met	Ser	Leu	Gly	Gly	Ala		
		115					120					125					
Ser	Gly	Ser	Thr	Ala	Met	Lys	Gln	Ala	Val	Asp	Asn	Ala	Tyr	Ala	Arg		
	130					135					140						
Gly	Val	Val	Val	Val	Ala	Ala	Ala	Gly	Asn	Ser	Gly	Asn	Ser	Gly	Ser		
145					150					155					160		
Thr	Asn	Thr	Ile	Gly	Tyr	Pro	Ala	Lys	Tyr	Asp	Ser	Val	Ile	Ala	Val		
			165					170						175			
Gly	Ala	Val	Asp	Ser	Asn	Ser	Asn	Arg	Ala	Ser	Phe	Ser	Ser	Val	Gly		
		180					185					190					
Ala	Glu	Leu	Glu	Val	Met	Ala	Pro	Gly	Ala	Gly	Val	Tyr	Ser	Thr	Tyr		
	195					200					205						
Pro	Thr	Asn	Thr	Tyr	Ala	Thr	Leu	Asn	Gly	Thr	Ser	Met	Ala	Ser	Pro		
	210					215					220						
His	Val	Ala	Gly	Ala	Ala	Ala	Leu	Ile	Leu	Ser	Lys	His	Pro	Asn	Leu		
225					230					235					240		
Ser	Ala	Ser	Gln	Val	Arg	Asn	Arg	Leu	Ser	Ser	Thr	Ala	Thr	Tyr	Leu		
			245					250						255			
Gly	Ser	Ser	Phe	Tyr	Tyr	Gly	Lys	Gly	Leu	Ile	Asn	Val	Glu	Ala	Ala		
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Ala Gln

<210> 4

<211> 269

<212> PRT

<213> Bacillus lentus

<400> 4

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His	Asn	Arg	Gly	Leu	Thr	Gly	Ser	Gly	Val	Lys	Val	Ala	Val	Leu	Asp		
		20						25					30				
Thr	Gly	Ile	Ser	Thr	His	Pro	Asp	Leu	Asn	Ile	Arg	Gly	Gly	Ala	Ser		
		35				40					45						
Phe	Val	Pro	Gly	Glu	Pro	Ser	Thr	Gln	Asp	Gly	Asn	Gly	His	Gly	Thr		
	50				55					60							
His	Val	Ala	Gly	Thr	Ile	Ala	Ala	Leu	Asn	Asn	Ser	Ile	Gly	Val	Leu		
65					70					75					80		
Gly	Val	Ala	Pro	Ser	Ala	Glu	Leu	Tyr	Ala	Val	Lys	Val	Leu	Gly	Ala		
			85					90					95				
Ser	Gly	Ser	Gly	Ser	Val	Ser	Ser	Ile	Ala	Gln	Gly	Leu	Glu	Trp	Ala		
		100						105					110				
Gly	Asn	Asn	Gly	Met	His	Val	Ala	Asn	Leu	Ser	Leu	Gly	Ser	Pro	Ser		
		115					120					125					

Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
 130 135 140
 Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser
 145 150 155 160
 Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
 165 170 175
 Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
 180 185 190
 Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
 195 200 205
 Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
 210 215 220
 Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
 225 230 235 240
 Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
 245 250 255
 Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
 260 265

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 <212> DNA
 <213> Artificial Sequence

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27

<210> 6
 <211> 27
 <212> DNA
 <213> Artificial Sequence

<220>
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<400> 6
 gtgtgtgggc cctattcgga tattgag

27

<210> 7
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 <212> DNA
 <213> Artificial Sequence

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<400> 7
 gtgtgtgggc ccatcagtct cacgacc

27